

Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats

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Abstract Fibrates are hypolipidemic drugs that activate the peroxisome proliferator-activated receptors. Since fibrates may also increase energy expenditure, we investigated whether fenofibrate (FF) had this effect in diet-induced obese rats. A 2-month administration of a high-fat palatable diet to adult rats increased body weight by 25% and white adipose mass by 163% compared with a standard diet. These effects were prevented by FF, both when administered for the 2 months of high-fat feeding and when given for only the second month. Consequently, FF-treated rats had a final body weight and white adipose tissue mass similar to untreated animals on the standard diet. FF also increased resting metabolic rate, hepatic peroxisomal and mitochondrial palmitoyl-dependent oxygen uptake and mRNA levels of acyl-CoA oxidase and lipoprotein lipase. Finally, FF lowered mRNA levels of uncoupling protein-2 and did not affect mitochondrial respiration in skeletal muscle. Therefore, FF seems to act as a weight-stabilizer mainly through its effect on liver metabolism. © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Fibrate; Obesity; Fatty acid; Peroxisome; Mitochondrion; Liver

1. Introduction

Fibrates are a class of drugs widely used to treat dyslipidemic patients, thus reducing the risk of developing coronary heart disease [1]. The molecular mechanism underlying the effect of fibrates has been elucidated [2]. Fibrates bind to and activate specific transcription factors belonging to the nuclear hormone receptor superfamily, the peroxisome proliferator-activated receptors (PPARs). These receptors form heterodimers with the retinoid X receptor and bind to peroxisome proliferator response elements in the promoter region of

target genes, whose transcription rate is consequently regulated [3,4]. Among the three principal isoforms so far identified (PPAR α , PPAR β and PPAR γ), PPAR α seems to mediate the hypotriglyceridemic effect of fibrates by inducing high rates of mitochondrial and peroxisomal β -oxidation in liver, kidney, heart and muscle [5,6] and by decreasing the plasma concentration of triacylglycerol-rich lipoproteins.

An intriguing aspect of fibrates is their potential role as regulators of energy homeostasis. It has been reported that fenofibrate (FF) does not affect body weight in Sprague-Dawley rats [7]. On the other hand, mice lacking PPAR α , are obese, no longer responsive to peroxisome proliferators and fail to induce the expression of genes required for fatty acid metabolism in mitochondria and peroxisomes [8,9]. In addition, there is evidence that FF can reduce body weight gain in animal models of diabetes, obesity and insulin-resistance such as fatty *falfa* Zucker rats and high-fat-fed C57Bl/6 mice [10,11].

Therefore, it might be possible that FF affects energy homeostasis only in the presence of an altered flux of fatty acids. It is well known that lipids accumulated in the adipose tissue largely derive from circulating triacylglycerols, especially during high-fat feeding. Thus, increased fatty acid oxidation in liver may play an important role in the regulation of body weight. In this scenario, the modulation of genes related to energy expenditure, such as uncoupling proteins (UCPs), may be involved. Recent evidence, in fact, indicates that, at least in mice, fibrates regulate UCP gene expression [12,13]. UCPs are mitochondrial carriers capable of dissipating the proton gradient across the mitochondrial inner membrane thus increasing thermogenesis while reducing the efficiency of ATP synthesis [14]. UCP-2 and UCP-3 may be relevant for the energy balance regulation because of their location in metabolically important tissues [15,16].

The aim of our study was to investigate whether FF increases energy expenditure, thus preventing body weight gain and increased adiposity, in diet-induced obese rats. We also verified whether FF reduces body weight gain after overweight had already been established in rats by high-fat feeding. In all animals we measured the gene expression of PPARs, their target genes and UCPs, as well as fatty acid oxidation in liver and in skeletal muscle. As both mitochondria and peroxisomes are very sensitive to fibrates [17,18], we extended the biochemical analyses to both organelles.

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; FF, fenofibrate; UCP, uncoupling protein; WAT, white adipose tissue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ACO, acyl-CoA oxidase; LPL, lipoprotein lipase; RT-PCR, reverse transcriptase-polymerase chain reaction

Table 1
Influence of FF on liver and visceral WAT weight and RMR

	C	D	DF1	DF2
WAT weight (g)	15.7 ± 1.9 (n = 4)	41.1 ± 3.5* (n = 4)	23.8 ± 7.4 (n = 4)	18.3 ± 3.5 (n = 4)
Liver weight (g)	14.9 ± 1.2 (n = 7)	18.7 ± 1.6 (n = 6)	25.3 ± 1.3** (n = 7)	26.8 ± 1.2** (n = 7)
RMR (l O ₂ /h/kg ^{0.75})	1.40 ± 0.06 (n = 4)	1.38 ± 0.11 (n = 4)	1.49 ± 0.09 (n = 4)	1.62 ± 0.12 (n = 4)

WAT, white adipose tissue. Results are presented as the mean ± S.E.M. **P* > 0.05 versus C, DF1 and DF2; ***P* > 0.05 versus C and D.

after 1 month of high-fat diet. At this time point, the body weight of DF1 rats was already significantly higher than C rats (439 g in DF1 and 380 g in C rats; +16%). The administration of FF for just 1 month to DF1 rats not only reduced body weight gain (490 g; −14% compared to D rats), but this effect was stronger in comparison to the DF2 group. In fact, during the last 30 days of the experiment the weight gain in DF1 rats was only 51 g as compared with 82 g in DF2 rats. In the same time frame, the body weight gain of D and C animals was 124 and 76 g, respectively.

As shown in Table 1 the visceral WAT mass was significantly increased in group D (+163%) when compared with group C (41.1 and 15.7 g, respectively). FF greatly reduced the WAT mass both when administered simultaneously and when administered after 1 month of high-fat diet (23.8 g in DF1 rats, −42% compared with D rats; 18.3 g in DF2 rats, −55% compared with D rats). When FF was administered to rats on a standard diet there was no significant effect on body weight gain or WAT mass (data not shown).

By daily monitoring, we did not observe any influence of FF on food intake (data not shown).

3.2. RMR

To verify if FF affects total body metabolic rate, we measured oxygen consumption in whole animals (Table 1). Resting oxygen consumption in group D was the same as group C (1.4 and 1.38 l O₂/h/kg^{0.75} b.w., respectively). RMR was increased in groups DF1 and DF2 compared with group D (+8 and +17%, respectively), although these differences are not statistically significant.

3.3. Peroxisomal and mitochondrial palmitoyl-dependent oxygen uptake

A high-fat diet did not affect the values of hepatic peroxisomal and mitochondrial palmitoyl-dependent oxygen uptake in D rats, while FF administration to DF1 and DF2 rats significantly increased both activities (Table 2). Mitochondrial respiration was increased by 82 and 106% in groups DF1 and DF2, respectively. The variations of peroxisomal respiration were much more marked. The increases were of about 24- and 38-fold in DF1 and DF2, respectively. No variations were found in skeletal muscle.

Table 2
Hepatic mitochondrial and peroxisomal palmitoyl-dependent oxygen uptake

	Mitochondria	Peroxisomes
C	5.37 ± 0.47	4.21 ± 1.1
D	5.01 ± 0.43	4.22 ± 1.5
DF1	9.10 ± 0.59*	101 ± 14.9*
DF2	10.33 ± 1.20*	161 ± 23.8**

The values are expressed as *n* atoms oxygen/min/mg protein of liver homogenate. Data are mean ± S.E.M., *n* = 4–5. **P* < 0.05 versus C and D. ***P* < 0.05 versus C, D and DF1.

The liver mitochondrial respiration rate, measured with succinate as substrate, did not show significant variations in state 4 and state 3 respiration rate among the groups studied (Table 3). Only a reduction (about −15%) in RCR was found in groups DF1 and DF2 compared with group D (6.3 and 6.1 versus 7.3, respectively). Similar results were obtained in skeletal muscle.

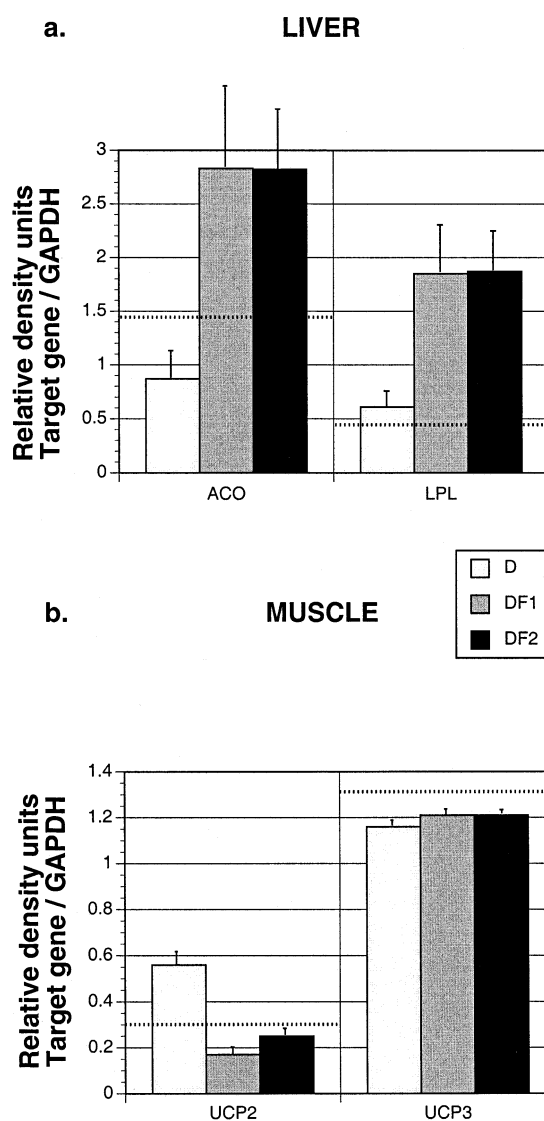


Fig. 2. RT-PCR analysis of gene expression in rat liver and skeletal muscle. Vertical bars represent the level of mRNA of different genes after normalization to the levels of GAPDH mRNA in the same samples. Dotted lines indicate the levels of mRNA in group C. Vertical lines indicate the S.E.M. Each group consisted of seven animals.

Table 3
Effect of FF on liver and gastrocnemius mitochondrial respiratory parameters

	Liver			Gastrocnemius		
	state 4	state 3	RCR	state 4	state 3	RCR
C	23 ± 2.0	170 ± 28	7.4 ± 0.7	85 ± 6.1	449 ± 35	5.3 ± 0.3
D	25 ± 2.8	183 ± 18	7.3 ± 0.6	71 ± 6.4	364 ± 28	5.3 ± 0.7
DF1	28 ± 2.0	179 ± 8.0	6.4 ± 0.1	92 ± 8.5	449 ± 35	4.9 ± 0.3
DF2	27 ± 2.0	164 ± 21	6.1 ± 0.6	90 ± 9.0	462 ± 46	5.1 ± 0.4

The values of state 4 and state 3 are expressed as ng atoms of oxygen consumption/min/mg of protein; RCR, respiratory control ratio (state 3/state 4 respiratory rates). Results are expressed as the mean ± S.E.M. of four experiments for each group. The differences among all groups were not statistically significant.

3.4. Gene expression

The level of expression of several genes was determined in liver and skeletal muscle (gastrocnemius) of rats belonging to the four experimental groups by RT-PCR. We measured mRNA of PPAR α , PPAR β , PPAR γ 1, PPAR γ 2, ACO, LPL, UCP-2 and UCP-3. There were no significant variations in the mRNA levels of PPARs in the animals treated with FF versus untreated animals, except for PPAR α , whose mRNA level was slightly increased in liver of DF1 and DF2 rats (1.7-fold; data not shown). On the contrary, 1 and 2 months of treatment with FF up-regulated the steady state levels of ACO mRNA (3.3-fold, DF1 or DF2 versus D) in liver (Fig. 2a). Also LPL mRNA levels were increased in liver of FF-treated rats receiving the high-fat diet (3.0-fold, DF1 or DF2 versus D) (Fig. 2a). UCP-2 was affected by FF only in muscle: mRNA levels decreased by 3.3- and 2.2-fold after 1 and 2 months of treatment, respectively (Fig. 2b). Conversely, muscle UCP-3 mRNA level did not change following FF administration (Fig. 2b).

3.5. Liver microscopic examination

As shown in Fig. 3, in the hepatocytes of D rats there is a lipid accumulation which is absent in hepatocytes of C rats. This accumulation disappeared following FF treatment as demonstrated in the hepatocytes of DF1 and DF2 rats.

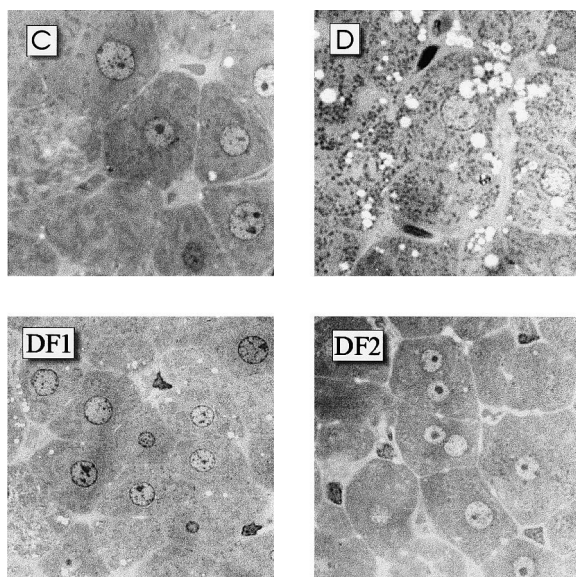


Fig. 3. Light microscopy of liver sections. White vacuoles represent lipid droplets. C, D, DF1 and DF2 indicate the different groups of rats as specified in the legend to Fig. 1.

4. Discussion

This study was undertaken to verify whether FF reduces body weight in normal rats on high-fat feeding and to obtain information about the cellular mechanism involved.

We observed that normal adult rats on a high-fat diet gained more weight compared with rats on a standard diet (final weights 571 and 456 g, respectively). This effect was not observed in an earlier study [19], however in our study the diet was given for a longer time (2 months versus 15 days) and our animals were older (60 versus 30 days). The obesity-like condition that we induced in rats mimics what occurs in humans on a high-fat diet in the early stages of obesity. In fact, the body weight gain of group D rats is largely due to an increase of the WAT mass, while the weight of other organs and tissues remained unchanged except for liver which showed steatosis and a 26% weight increase.

An important finding is that FF reduced the weight of rats fed the high-fat diet for 2 months, compared with sex- and age-matched untreated rats receiving the same diet. This was also true for those animals who started FF 1 month after the beginning of the high-fat diet. Interestingly, the final weight of both groups of treated animals was very similar to that of untreated animals on the standard diet. This indicates that, in the presence of fat accumulation, FF not only prevents excessive weight gain, but is also able to mobilize fats from adipose tissue depots. It is also likely that both the prevention of fat accumulation and fat mobilization in WAT are driven by the increased fat catabolism in liver. In fact, in FF-treated rats, the liver was even heavier than in group D rats, but hepatocytes did not contain lipid droplets in contrast to hepatocytes from group D rats. This finding is in accordance with the observation of liver hypertrophy after FF administration, due to proliferation of peroxisomes and mitochondria. Peroxisome respiration was greatly increased in DF2 rats (38-fold compared to C or D rats), which confirms that FF induces marked peroxisome proliferation. Similarly, mitochondrial respiration was almost doubled in DF1 and DF2 animals. Moreover, although the respiration of purified mitochondria was unchanged (see Table 3), mitochondrial respiration in liver homogenate was significantly increased (see Table 2), which indicates that the enhancement was due to an increased number of mitochondria. It is noteworthy to report that the increase in liver activity was paralleled by a large reduction in WAT mass, which can account for most of the body weight reduction. Also the increase in RMR of FF-treated rats (+8 and +17% in DF1 and DF2, respectively) may be accounted for by the increase in hepatic mitochondrial and peroxisomal β -oxidation. In fact, it is well known, that the liver contributes for about 10–20% to the RMR. There-

fore, a 100% increase of oxygen consumption by the liver would explain an increase of RMR of the order of 8–17%.

To gain insight into the molecular mechanisms underlying the effects described above, we measured the mRNA levels of PPARs because self-regulation of nuclear receptors by ligands is reported [29,30] and FF binds strongly, although not exclusively to PPAR α . However, except for a slight 1.7-fold increase of PPAR α liver expression in FF-treated rats, no variations in the expression of other PPAR isoforms were observed in the different experimental conditions. We then investigated genes involved in lipid metabolism and energy expenditure (LPL, ACO, UCP-2 and UCP-3) and found that LPL and ACO gene expression was up-regulated by FF treatment in the presence of the high-fat diet. Although the combined action of FF and the high-fat diet was not known, the effect on ACO and LPL expression was partly expected, since these are targets of FF-activated PPARs [4], and thus provide a positive control of the experimental conditions. However, the association of the activation of these genes and weight reduction is a novel finding. Surprisingly in our case, UCP-2 expression was not induced in the liver of D rats where the number of lipid droplets was increased; FF did not alter this situation. Differently, UCP-2 expression may be induced by lipid substrates in isolated hepatocytes [31]. These apparently contrasting results may be explained by the differences between our *in vivo* experimental setting and the *in vitro* one used by Cortez-Pinto et al. [31]. In contrast to liver, UCP-2 expression in skeletal muscle was stimulated by the high-fat diet and inhibited by FF. If UCP-2 is involved in lipid metabolism, this result may be in accordance with the increased fatty acid influx and utilization in muscle consequent to a hyperlipidic diet; vice versa, when β -oxidation in liver is up-regulated less fatty acids are available to muscles even when the diet contains large amounts of lipids. Finally, we did not observe variations in UCP-3 mRNA level in the muscle of rats that were on the high-fat diet alone or in combination with FF treatment. Interestingly, it has been reported that UCP-3 expression in rat muscle is increased after fasting, a condition of altered energy intake different from high-fat feeding [32]. Thus, FF may exert an indirect effect in muscle, which may be related to its action in liver.

In conclusion, we provide evidence that FF acts as a 'weight-stabilizer' through enhancement of lipid catabolism in rat liver. This effect is mediated by PPARs, mainly through the induction of target enzymes involved in hepatic lipid metabolism. Although both the peroxisomal and the mitochondrial compartments contribute significantly to the increased oxidation of fatty acids, our data do not support a role of mitochondria in wasting energy, which is instead an intrinsic property of peroxisomal β -oxidation. Skeletal muscle does not seem to play a relevant role in dissipating energy, thus indicating the liver as the major player in the effects exerted by FF on body weight and WAT mass in rodents.

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